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(215) 233-6400

Title: Computer-Controlled Microwave Heating to in-Package Pasteurize Beef Frankfurters for Elimination of *Listeria monocytogenes*

Author(s): L. Huang,

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COMPUTER-CONTROLLED MICROWAVE HEATING TO IN-PACKAGE PASTEURIZE BEEF FRANKFURTERS FOR ELIMINATION OF *LISTERIA MONOCYTOGENES**

LIHAN HUANG[1]

U.S. Department of Agriculture
Eastern Regional Research Center
Agricultural Research Service
600 E. Mermaid Lane, Wyndmoor, PA 19038

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ABSTRACT

The objective of this study was to develop an in-package pasteurization technology to kill Listeria monocytogenes *in ready-to-eat meats using microwave heating. This technology utilized an infrared sensor to monitor the surface temperature of beef frankfurters during microwave heating. The aim was to increase the surface temperature of frankfurters to a set point lethal to* L. monocytogenes. *A feedback control mechanism was used to control the power to the microwave oven. Results indicated that the simple on-off control mechanism was able to maintain the surface temperature of beef frankfurters near the respective set points of 75, 80 or 85C used in this study. This pasteurization process was able to achieve a 7-log reduction of* L. monocytogenes *in inoculated beef frankfurters using a 600-W nominally rated microwave oven within 12–15 min. If optimized, this system may provide the food industry with a terminal, postlethality pasteurization technology to kill* L. monocytogenes *in ready-to-eat meats within the final packages.*

INTRODUCTION

Listeria monocytogenes is a deadly pathogen that has caused many incidents of fatalities in recent multiple-state outbreaks of listeriosis associated with ready-to-eat (RTE) meat and poultry products in the United States (Centers for Disease Control and Prevention [CDC] 1998, 2000, 2002). This

* Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

[1] Corresponding author. TEL: (215) 233-6552; FAX: (215) 233-6559; EMAIL: lhuang@errc.ars.usda.gov

bacterium is Gram-positive, rod-shaped, facultative and widely distributed in the environment, including soils, water, sewage and animals. Human listeriosis is relatively rare because this organism poses very little risk to healthy people with normal immune systems. However, this organism can be life-threatening to the population with compromised immune systems. In general, the elderly, pregnant women, fetuses and neonates are particularly susceptible to *L. monocytogenes*. The mortality rate can be as high as 20% among this segment of the population. In all the three multiple-state outbreaks of listeriosis reported by the CDC (1998, 2000, 2002), fatality, miscarriage or stillbirth occurred. *L. monocytogenes* has been isolated in a variety of foods, including raw and processed meat and poultry products, seafood, salads and unpasteurized dairy products. RTE meat products, such as frankfurters and deli meats, are frequently contaminated with *L. monocytogenes* (Ryser and Marth 1991).

This organism can grow under refrigerated conditions (Ryser and Marth 1991). Therefore, RTE products contaminated with *L. monocytogenes* may present a real danger for consumers susceptible to this organism because products in this category, by convention, may be consumed directly without any further cooking or reheating. Because of the high risk of this organism to the general public, the U.S. Food and Drug Administration (FDA) has adopted a "zero tolerance" policy for this organism in RTE foods. Under this policy, no *L. monocytogenes* should be detected in either of two 25-g samples of RTE foods. Similarly, the Food Safety and Inspection Service (FSIS) of the USDA also has maintained a policy of "zero tolerance" for *L. monocytogenes* in RTE meat and poultry products regulated by this agency (FDA/FSIS/CDC 2003).

Manufacturing of RTE meats involves a cooking/thermal processing process designed to eliminate foodborne pathogens (including *L. monocytogenes*) in the products. Therefore, RTE meat products are supposedly free of *L. monocytogenes* immediately after cooking or thermal processing. However, postprocessing recontamination can occur in the production facilities by construction, failure to control sanitation procedures, employee hygiene, movement of supplies and products or other entry vector (USDA/FSIS 2004). Postprocessing operations also can contribute to the cross-contamination. According to a joint study conducted by scientists from the CDC and USDA in a turkey franks manufacturing facility, contamination of *L. monocytogenes* was primarily found in the peeling process of fully cooked turkey franks immediately before the final packaging of products (Wenger *et al.* 1990). As a result, RTE meat products contaminated with *L. monocytogenes* may enter the market, potentially causing outbreaks of listeriosis. To eliminate the risk of listeriosis caused by *L. monocytogenes* in RTE meats, the FSIS strengthened its regulations in an effort to reduce *L. monocytogenes* in RTE meat and poultry products in 2003 (Federal Register 2003). The new rules require the manufacturers to adopt one of three alternatives to ensure the safety of RTE

products. The alternatives include: (1) Employ both a postlethality treatment and a growth inhibitor for *Listeria* on RTE products. Establishments opting for this alternative will be subject to FSIS verification activity that focuses on the postlethality treatment effectiveness. (2) Employ either a postlethality treatment or a growth inhibitor for *Listeria* on RTE products. Establishments opting for this alternative will be subject to more frequent FSIS verification activity than for the previous alternative. (3) Employ sanitation measures only. The alternative will receive the most frequent level of FSIS verification activity.

Both low temperature steam and hot water have been tested for postprocessing in-package pasteurization of cooked meats (Murphy *et al.* 2001, 2003a,b; Muriana *et al.* 2002; Murphy and Berrang 2002a,b). In these studies, fully cooked, vacuum-packaged poultry products were exposed to a heating medium, either steam or hot water, at temperatures above 88C to inactivate *L. monocytogenes, Listeria innocua* or *Salmonella* Sentfenberg. Murphy *et al.* (2001) and Murphy and Berrang (2002b) reported that the heating time needed to achieve a 7-log reduction of *S.* Senftenberg and *L. innocua* in fully cooked chicken-breast strips (454 g) was 34 min for a continuous process, or 40 min for a batch process in a 88C steam environment. Murphy and Berrang (2002a) used a water-immersion process (88C) to kill *S.* Senftenberg and *L. innocua* in fully cooked chicken-breast strips packaged in two different-sized vacuum packages (227 or 454 g). They reported that a 20-min heating was needed to achieve a 7-log reduction of *S.* Senftenberg and *L. innocua* in 227-g packages, while 34 min was needed in 454-g packages. Muriana *et al.* (2002) reported that a 2-log reduction of *L. monocytogenes* in deli meats could be achieved during the first 2-min hot-water immersion at temperatures between 195 and 205F. The bacterial reduction was between 2 and 4 logs in a 10-min water-immersion process. Murphy *et al.* (2003b) further reported that postcook in-package pasteurization with hot water was highly dependent upon the thickness of products.

Microwave heating utilizes electromagnetic energies at frequencies of 915 MHz or 2450 MHz to generate heat in materials (Decareau and Peterson 1986). Microwave energy can penetrate food materials and produces volumetrically distributed heating effects, resulting from molecular friction within foods (Oliveira and Franca 2002). Therefore, it has been used as a heating source to kill microorganisms in foods (Holyoak *et al.* 1993; Gundavarapu *et al.* 1995; Heddleson *et al.* 1996). More recently, Guan *et al.* (2003) demonstrated the feasibility of using a 915-MHz pilot-scale microwave heating system to inactivate spores of *Clostridium sporogenes* (PA 3679, NFPA NO. SC 218) in macaroni and cheese products. However, using microwave heating for in-package pasteurization of RTE meat to kill *L. monocytogenes* has not been reported in the literature.

The objective of this study was to develop a postlethality processing technology to pasteurize RTE meats after they are packaged. Such a technology would enable the destruction of *L. monocytogenes* within the packages of RTE meats without further contamination if the packages remain intact prior to final consumption. The main goal was to demonstrate the feasibility of this technology by developing a microwave heating system with a feedback control mechanism to monitor and control the process of in-package pasteurization for RTE meats. The ultimate goal of the research was to ensure the safety of RTE meat and poultry products.

MATERIALS AND METHODS

Sample Preparation

Frozen beef frankfurters were purchased from a local manufacturer. Each frankfurter was approximately 2.2 cm in diameter and 13.3 cm in length. The frankfurters were manufactured in the same batch without adding any antimicrobial agents. Upon receiving, samples were kept frozen (≈–20C) in a freezer until ready for use in experiments. Frozen frankfurter samples were thawed overnight in a refrigerator (≈4C) prior to being used in the experiments. Frankfurters are very susceptible to heat treatment, and can be easily damaged by heating. However, because frankfurters were easy to obtain, they were chosen for testing the concept of in-package pasteurization by microwave heating.

Bacterial Strains

Four *L. monocytogenes* strains (H7763, H7776, H7778 and 46877) were used in this study. The first three strains (H7763, H7776 and H7778) were actual outbreak strains of *L. monocytogenes* from hot dogs, while the last strain (46877) was an outbreak strain from fresh pork sausage. All these strains of *L. monocytogenes* were obtained from Dr Vijay K. Juneja's lab of Microbial Food Safety Research Unit of the USDA's Agricultural Research Service, Eastern Regional Research Center (ARS ERRC) located at Wyndmoor, PA.

The stock cultures of each strain of *L. monocytogenes* were prepared by growing them at 37C overnight (24 h) in 10 mL Brain Heart Infusion broth (BHI broth, BD/Difco Laboratories, Sparks, MD). Each strain was transferred onto trypticase soy agar (TSA, BD/Difco Laboratories, Sparks, MD) and then incubated at 37C FOR 18 h. This became the stock culture for each strain of *L. monocytogenes* used in this study. The stock cultures were periodically renewed by repeating the same procedures described in this section.

Bacterial Cultures

One day before conducting the pasteurization study, each strain of the bacteria was transferred to 25 mL of BHI broth and incubated at 37C for approximately 24 h in an orbital shaker. The bacterial cells were spun down in a refrigerated centrifuge (Model Marathon 21000R, Fisher Scientific Co., Pittsburgh, PA) operated at 2400 × g for 15 min at 4C. Each culture was washed once with 10 mL of 0.1% peptone-water and centrifuged again. Pellets of each strain were combined and resuspended in 20 mL of sterile 0.1% peptone-water. The culture mixture, containing $\approx 10^{9.5}$ cfu/mL, was maintained on ice before being inoculated onto to frankfurter samples.

Thermal Resistance of *L. monocytogenes*

To evaluate the process of thermal inactivation of *L. monocytogenes* in frankfurters, thermal resistance of this organism was measured. Frankfurter samples, cut into 5 ± 0.05-g segments, were placed into sterile plastic filter bags (12 × 19 cm, Model BagPage BP 100, Topac Inc., Hingham, MA). The bacteria cocktail (0.1 mL at $\approx 10^{9.5}$ cfu/mL) was directly added to the 5-g sample in each filter bag. The bacterial culture and sample were manually mixed, and then the sample was flattened into a thickness of 1 mm with a round bottle. Each bag was vacuumed to remove air from the plastic filter bag and sealed under a vacuum level of about 700 mmHg.

Each bag containing the 5-g sample was immersed in a circulating water bath (IsoTemp 1016S, Fisher Scientific, Inc., Pittsburgh, PA) maintained at 57, 60, 63 or 66C. During each experiment, a thin thermocouple (Type T, 40 AWG, Omega Engineering, Inc., StamFord, CT), was inserted into the sample with the insertion area sealed with heat-stable high-vacuum silicone grease (Dow Corning Corp., Midland, MI). This thermocouple measured the temperature of the sample inside the plastic bag. The average come-up time was measured as 15 s, which was included as part of the heating time.

At time intervals appropriate to the heating temperature, samples were removed from the water bath and immediately submerged into an ice/water slurry. After each heating experiment was completed, 5 mL of sterile 0.1% peptone-water was added to each bag. The sample was then stomached in a MiniMix Stomacher (Model BagMixer 100 W, Interscience Co., St. Nom, France) at the maximum speed for 90 s to completely homogenize the sample. After stomaching, the clear portion (0.1 or 1 mL) of the mixture in each bag was serially diluted and surface-plated onto PALCAM *Listeria* selective agar (Difco/BD) (van Netten *et al.* 1989). The plates remained at room temperature for about 2 h to allow the recovery of the heat-injured cells. After that, the plates were incubated at 37C for 48 h. Typical *Listeria* colonies were counted

after incubation. The bacterial counts were converted to the logarithm of colony-forming unit per g or log (cfu/g). For thermal inactivation analysis, each experiment was replicated twice.

For each heating temperature, the surviving bacterial counts (log[cfu/g]) were plotted against time. With the first and last data points excluded, the linear portion of each survivor curve was used in linear regression to calculate the slope. The D value at each heating temperature was calculated from the inverse of each survivor slope. The logarithm of the D values under different temperatures was plotted against temperature to calculate the Z value of *L. monocytogenes* in frankfurters used in this study.

Sample Inoculation

Immediately before the experiments, samples were retrieved from the refrigerator and placed into plastic bags (0.4 mm thick, 15.2 cm × 35.6-cm standard gas/moisture barrier bags). Each plastic bag contained five frankfurters arranged in parallel in a single layer. With the frankfurters inside the plastic bags, samples were held in a vertical position. The cocktail (0.1 mL) of bacterial culture solution was applied onto one of the folded ends of each frankfurter. The samples were maintained in the vertical position to allow the bacterial solution to flow across the entire length of each frankfurter. After that, each frankfurter was rotated and rubbed gently against each other inside the plastic bags to allow the bacteria to spread over the entire surfaces. The samples were vacuumed to a final level of approximately 700 mmHg and sealed. Samples were then maintained in ice for approximately 1 h before being subject to microwave pasteurization.

Microwave Heating and Instrumentation System

A generic-brand small household microwave oven was purchased for this study from a local retail store. The oven was rated nominally at 600 W and was equipped with simple mechanical controls for heating time and power levels. Although the microwave oven was nominally rated at 600 W, the average power output from the magnetron inside the microwave oven (Model 2M213 Series, LG Electronics, Inc., Seoul, Korea) was actually rated at 550 W. Its filament was operated at 3.5 Vac with 0 preheating time. The frequency generated by the magnetron was 2450 MHz. It was also equipped with a turntable to allow uniform heating of foods.

To convert the microwave oven into a computer-controlled system for pasteurizing frankfurters, a hole of 1.5 in. (3.81 cm) in diameter was drilled through the center of the top wall of the oven chamber (Fig. 1). Through the hole was installed a stainless steel pipe of the same diameter with a length

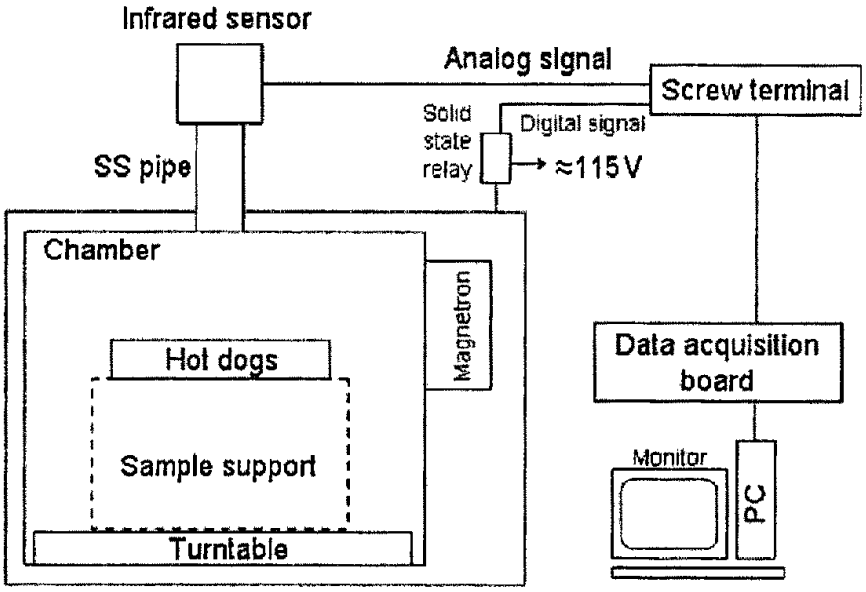

FIG. 1. COMPUTER-CONTROLLED MICROWAVE HEATING AND INSTRUMENTATION SYSTEM

of 6 in. (15.2 cm). Immediately on top of the stainless steel pipe, an infrared pyrometer (Model OS65-V-R5, Omega Engineering, Inc., Stamford, CT) was installed. The purpose of installing a stainless steel pipe between the oven wall and the infrared sensor was to prevent microwaves from directly hitting the sensing head of the infrared sensor. Measurement of the electromagnetic fields in the position of the infrared sensor confirmed that no microwave signals were detected using an RF Field Strength Meter (AlphaLab, Inc., Salt Lake City, UT).

The infrared sensor was used to measure the surface temperature of frankfurter samples during microwave heating. This model of the infrared sensor had a clear sensing aperture of 2.54 cm and a field of view (FOV) ratio of 15:1. It had a straight focused planar view of 2.54 cm in diameter up to 38 cm away from the sensing head. It produced a voltage signal (0–5 V) when being used to measure temperatures. Frankfurter samples were located approximately 30 cm below the sensing head of the infrared sensor.

A high-precision temperature/voltage PCI data acquisition board (Model NI4351, National Instruments Corp., Austin, TX) was used to measure the voltage signals from the infrared sensor. This data acquisition board had a 24-bit analog-to-digital conversion resolution and eight digital I/O lines. It was

interfaced with the infrared sensor through a connector block (Model TBX-68T, Model NI4351, National Instruments Corp., Austin, TX) and a shielded cable (Model SH6868, Model NI4351, National Instruments Corp., Austin, TX).

Computer-controlled Microwave Heating

A plastic shelf (20 cm × 10 cm × 10 cm), modified from standard culture tube racks and transparent to microwaves, was used to support the sample during microwave heating. The support was used to raise the position of the sample to the geometric center of the oven, allowing microwaves to hit the sample from both the top and bottom directions. The center of the flat surface of the sample was positioned directly below the infrared sensor. As the turntable moved during microwave heating, the sample, located on top of the plastic shelf, rotated with the turntable along the axis of the infrared sensor. During microwave pasteurization, the infrared sensor measured the average temperature of a round spot of 2.54 cm in diameter at the center of the sample surface at a 1-s interval.

A computer program was developed to monitor and control the process of in-package pasteurization. This program was designed using a graphical computing language (LabView Version 6.0, National Instruments Corp., Austin, TX) and was used to provide a feedback control of the operations of the microwave oven. This program utilized the average of the two consecutive measurements of the surface temperature as a control signal. The control program was based on two schemes (Fig. 2). In the first scheme, the infrared sensor measured the surface temperature of a sample being exposed to microwaves in the oven chamber. This voltage signal was compared to the voltage set point (V_{set}), corresponding to the respective pasteurization temperature set point. At this stage of the pasteurization, the surface temperature of the sample was always below the temperature set point. Therefore, a signal was sent from a digital port of the data acquisition board to the solid relay, allowing the electric power to be connected to the microwave oven continuously.

As soon as the surface temperature was above the set point, the second control scheme began to function. The second control scheme allowed the sample to be held around the set point for a specific amount of hold time, while the power to the microwave oven was turned on or off according to the difference between the measured voltage and the set point.

The feedback control mechanism provided a means to monitor and adjust the release of microwave energy to the food sample being heated by the microwave oven. It could prevent the food samples from overheating inside the microwave oven, while allowing their surface temperatures to be held around the set point for an extended period of time. This enabled the microwave

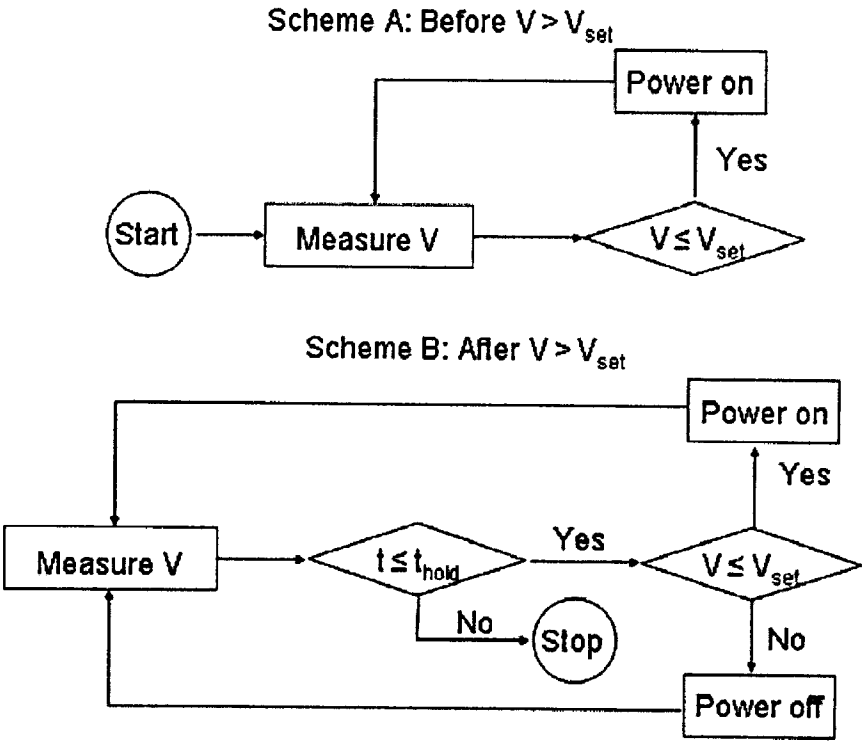

FIG. 2. CONTROL SCHEMES FOR MICROWAVE PASTEURIZATION OF BEEF FRANKFURTERS

energy to penetrate into the interior of the samples, raising their temperatures from within and killing the bacteria in the frankfurters. Because of the small size of the microwave oven, only one sample (five frankfurters in a plastic bag) was treated each time.

Three surface temperature set-points (75, 80 and 85C) were used, with only one temperature set point applied during each experiment. The hold time for each temperature set point is listed in Table 1. Each combination of temperature and hold-time was replicated three times.

Calibration of the Infrared Sensor

The infrared sensor used in this study was calibrated before it was used for temperature measurement and control. To calibrate the infrared sensor, a Type-T thermocouple was attached onto the surface of a plastic bag containing five frankfurter samples using a small drop (≈1 mm in diameter) of superglue. The wire size of the thermocouples used in this study was 44 Ga or 0.05 mm

TABLE 1.
HOLD TIME AT EACH TEMPERATURE SET-POINT

Temperature set point (C)	Average come-up time (s)*	Additional hold time (s)
75	125	200, 400, 600, 700, 800
80	133	175, 350, 525, 660, 700
85	138	150, 300, 450, 600

* Average come-up time is the time immediately when the surface temperature of a sample was raised to a set point.

in diameter. The response time of the thermocouples was about 5 ms, according to the manufacturer. During calibration of the infrared sensor, the thermocouple actually measured the surface temperature of the plastic bag containing five frankfurters. Because the thickness of the plastic bag was very small, and because it was tightly in close contact with the frankfurters because of the vacuum applied to the package, the difference between the surface temperature of the plastic bag and the surface temperature of the frankfurters was ignored. Therefore, the surface temperature of the plastic bag was treated as the surface of frankfurters inside the plastic bags.

The sample with the thermocouples was fully submerged in a circulating water bath maintained at 90C for 30 min. After that, the sample was taken out from the water bath and placed directly underneath the infrared sensor. The distance between the sample and the infrared sensor was about 25.4 cm. After the water residue on the surface of the plastic bag was removed with paper towels, the surface temperature of frankfurters and the voltage of the infrared sensor were measured simultaneously as the sample cooled down naturally. The voltage signal from the infrared sensor was then correlated to the surface temperature of frankfurters.

Internal and Surface Temperature Measurements by Fiber-optic Probes

Because the infrared sensor used in this study could only measure the surface temperature of frankfurters subjected to microwave heating, independent experiments were conducted to measure the internal temperature. To accomplish this, a fiber-optic probe (SIW-02, Luxtron Corp., Santa Clara, CA) was inserted through the plastic bag, and sandwiched between two middle frankfurters. A thin rubber band was used to fix the fiber-optic probe along the middle contact line between the frankfurters. The position of the sensing tip was about one-half of the length of a frankfurter. After the area where the probe was inserted through the plastic bag was sealed with sufficient amount of heat-stable high-vacuum grease (Dow Corning Corp., Midland, MI), the sample (containing five frankfurters) was sealed. The grease was applied both

inside and outside of the insertion area and was inserted to seal the insertion hole. After the sample was sealed, all five frankfurters in a single layer were tightly pressed against each other. The sample was then subject to the same microwave heating conditions. The only exception was that the turntable was disabled. The fiber-optic probe was connected to a fluoroptic thermometer (Model 790, Luxtron Corp., Santa Clara, CA). The voltage output signals from the fluoroptic thermometer were sent to the data acquisition board mentioned previously. The temperature data were collected at the same interval. Throughout the experiments the vacuum was maintained without any leakage. Under the vacuum, the five frankfurters were tightly packed in the plastic bag, and therefore the five frankfurters can be treated as a rectangular block. This probe measured the temperature at the geometric center of the five tightly packed frankfurters.

Another set of experiments was conducted to measure the surface temperature using the fiber-optic probe. The preparation was identical to the procedures described previously, except that the probe was attached onto the surface of the middle of the five frankfurters. The surface and internal temperatures were measured independently because of the difficulty of maintaining the vacuum when both probes were inserted at the same time. To reduce the amount of experiments, the surface temperature set-point was set to 75C when measuring the surface and internal temperatures by fiber-optic probes. The total heating time was 900 s.

Microwave Pasteurization of Frankfurters

Before each experiment, the surface temperature set point and the hold time was inputted into the control program. The sample was taken out of ice, quickly wiped with dry paper towels to remove ice and water residue and then loaded onto the plastic shelf inside the microwave oven. The computer-control program was immediately activated, and the pasteurization process was initiated.

After microwave heating, the heated sample was immediately taken out of the microwave oven, and dropped into an ice slurry. The sample was maintained in the ice slurry for at least 20 min to allow it to cool down. The packages of all samples were intact after microwave heating.

Determination of Bacterial Counts

After the samples were sufficiently cooled down, each was dipped into a Clorox bleach solution containing approximately 0.5% v/v hypochlorite. Each package was cut open with a pair of sterile scissors. Three frankfurters, located from the center to the edge inside each plastic bag, were removed for

determining the surviving bacteria. Each frankfurter was dropped into a filter bag (SFB-0410, Spiral Biotech., Inc., Bethesda, MD) containing 150 mL of 0.1% sterile peptone-water. Each sample was stomached in the filter bag using a stomacher (Seward Laboratory Stomacher 400, London, U.K.) for 90 s. After that, a small volume, 100 or 1000 μL, depending on the bacterial counts, was extracted from the liquid portion of the homogenate, serially diluted and then spread-plated onto the surfaces of TSA. For samples with low bacterial counts, the homogenate (1000 μL) was directly plated onto TSA. Duplicated plates were used for each frankfurter per dilution. The TSA plates were maintained at room temperature for approximately 3 h to allow for the recovery of heat-injured cells of *L. monocytogenes*.

After that, approximately 10 mL of freshly prepared PALCAM *Listeria* selective agar was overlaid onto each TSA plate. The PALCAM *Listeria* selective agar allowed the surviving *L. monocytogenes* to grow, while suppressing the growth of natural flora in the samples. The PALCAM/TSA overlay plates incubated at 37C for approximately 48 h. Typical *Listeria* colonies were counted. The plate counts were converted to the logarithms of bacterial counts per g of frankfurters. Because the frankfurters were obtained from the same batch, the weight of each frankfurter was fairly uniform. The weight of 20 frankfurters was 44.6 ± 0.1 g (mean ± SE). This value was used as the weight of each frankfurter during calculation and conversion of bacterial counts. Preliminary experiments showed the use of TSA in combination with PALCAM *Listeria* selective agar could recover more *L. monocytogenes* from heat-treated frankfurters.

For the convenience of presenting data, a bacterial count was treated as 0 log (cfu/g) if no viable cells of *L. monocytogenes* were recovered from pasteurized samples. Having a 0 log (cfu/g) only indicates that the detection of bacteria was very low and beyond the limit of method used in this study.

Thermal Process Analysis

The temperature history measured at the geometric center of the frankfurter packages was used to analyze the process of microwave thermal pasteurization. Based on the D and Z values determined using the method mentioned previously, the general method was used to calculate the total lethality during the pasteurization process. The total lethality, or the total log-reduction (TLR), can be calculated from:

$$\mathrm{TLR} = \frac{1}{D_{\mathrm{ref}}} \int_0^t 10^{\frac{T-T_{\mathrm{ref}}}{Z}} \, dt \tag{1}$$

In the previous equation, T_{ref} is the reference temperature, and D_{ref} is the D value at the reference temperature. To use the general method, the reference temperature used in this study was 70C. Eq. (1) was solved using the trapezoidal rule.

RESULTS AND DISCUSSION

Thermal Resistance of *L. monocytogenes*

The inactivation of *L. monocytogenes* in beef frankfurter samples under isothermal conditions generally follows a first-order kinetics (Fig. 3A) for all temperatures. A linear relation between the logarithm of D values and heating temperature was also observed (Fig. 3B). The Z value of this organism calculated from the slope of Fig. 1B was 5.47C, or:

$$\log(D) = 13.3 - \frac{T}{5.47}. \quad (R^2 = 0.997) \tag{2}$$

To analyze the thermal inactivation process, a reference temperature of 70C was used. The D value at 70C, or D_{70C}, calculated from Eq. (2) was 3.2 s.

Calibration Curve of Infrared Sensor

Figure 4 shows the calibration curve of the infrared sensor used in this study. The raw data were gathered from five independent experiments. Because of the immediate cooling after the samples were removed from the water bath, the achieved highest surface temperature was about 80C. Because the samples were held at room temperature during calibration, the sample surface temperature decreased exponentially with time. As the surface temperature approached room temperature (around 20C), the cooling process was very slow. However, it was not necessary to gather the data around room temperature because it could not significantly improve the accuracy of the calibration. Therefore, the data gathering was terminated as the surface temperature was between 25 and 30C.

The signals from the infrared sensor responded linearly with the surface temperature of the samples. According to the calibration curve, the signal from the infrared sensor changed approximately 0.02 V per degree C of temperature. Although the emissivity of the sensor could be adjusted, it was not necessary to do so because the calibrated raw voltage signal was directly used in the control process. Because no data were gathered above 80C or below 25C, extrapolation was used for temperatures beyond these limits.

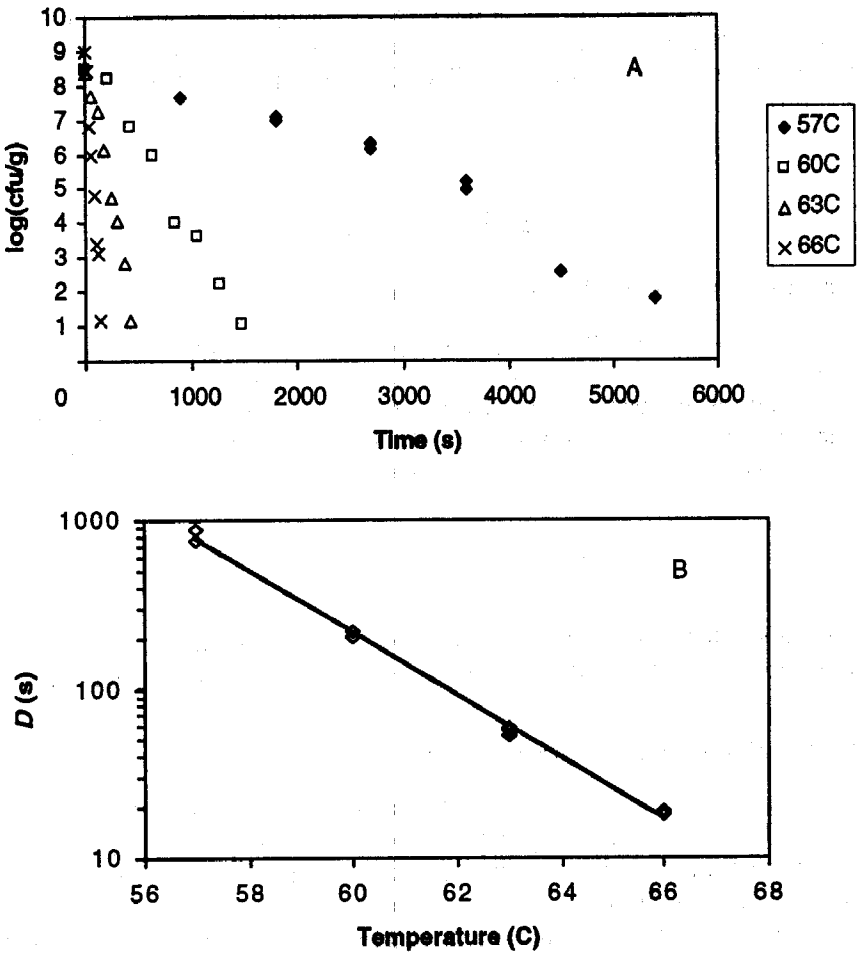

FIG. 3. THERMAL INACTIVATION KINETICS OF *LISTERIA MONOCYTOGENES* IN BEEF FRANKFURTERS
(A) Thermal inactivation curves. (B) Linear relationship between the logarithm of *D* values and temperature.

Computer-controlled Microwave Heating

As a sample was exposed to the microwave field, its surface began to absorb the energy from the microwaves. With the microwave energy directly absorbed by the frankfurters, the thermal energy would also travel by conduction into the interior. The internal heating was a net result of both the absorption of microwave energy and the conduction of heat after the microwave energy was converted to thermal energy, leading to an increase in the sample

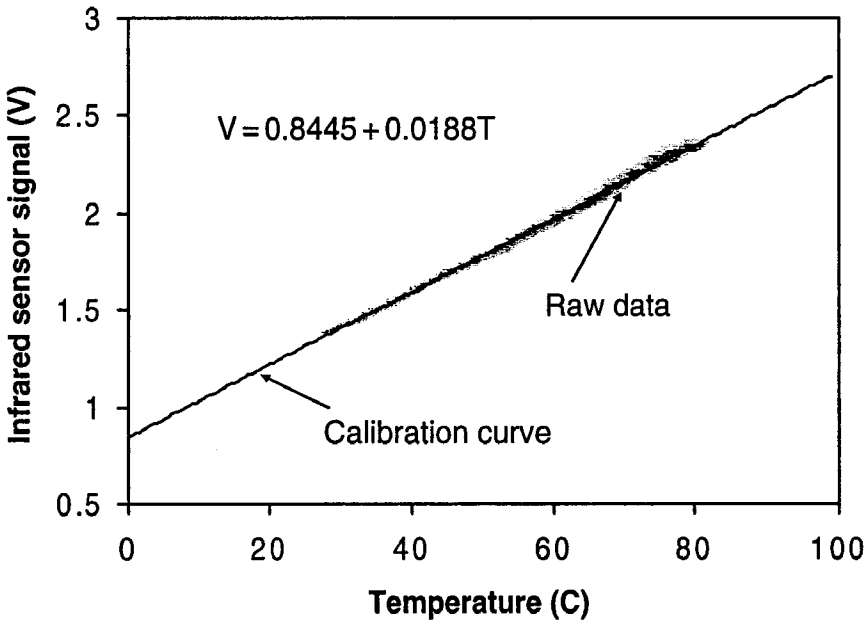

FIG. 4. THE INFRARED SENSOR CALIBRATION CURVE

temperature. If the sample was exposed to the microwave energy without any limitation, its temperature would continue to grow. If the internal pressure of the sample exceeded the physical strength of the plastic packaging material, both sample and the packaging material may explode. Because of the feedback control mechanism implemented to monitor the process of microwave heating, explosion never occurred in this study.

Figure 5 shows the temperature histories on the surface of samples subjected to microwave heating. The average initial temperature of the samples was 7.3 ± 0.2C. Apparently, the surface temperature of the samples did not rise to the set points immediately when they were exposed to the microwave fields. The average come-up time, or the time needed to raise the surface temperature to a set point, was 125 ± 2.7, 133 ± 2.5 or 138 ± 1.9 s for the set point of 75, 80 or 85C during microwave heating of a pack of five beef frankfurters (≈220 g).

Before reaching the set points, the surface temperature fluctuated as it increased. The temperature fluctuation during come up was probably caused by the uneven distribution of the microwave fields within the oven. After the surface temperature was raised above the set point, the second scheme of on-off feedback control mechanism began to function. The control program cut off or turned on the power supply of the oven, depending on the difference

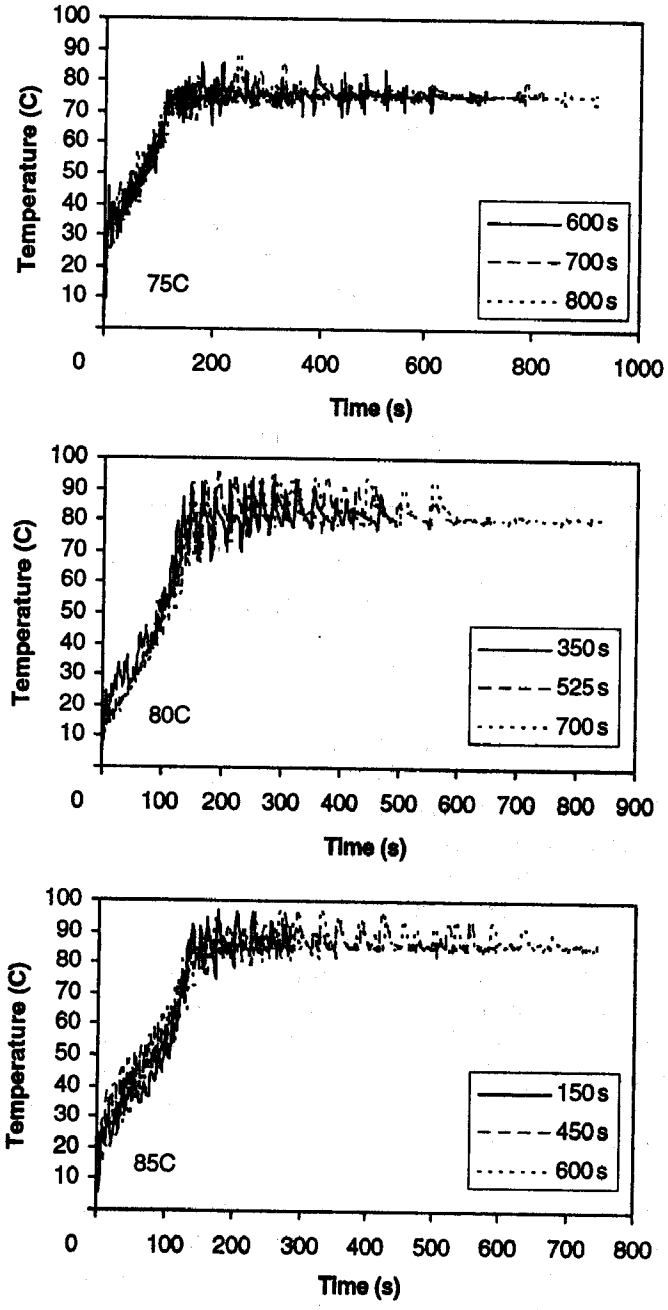

FIG. 5. THE SURFACE TEMPERATURE CURVES DURING COMPUTER-CONTROLLED MICROWAVE HEATING TO 75, 80 AND 85C
The legends in the graphs are hold time after the surface temperature reached the set points.

between the set point and the surface temperature of the sample. Although overshooting occurred, the surface temperature oscillated around the set point of each process. Judging from Fig. 5, the feedback control mechanism was able to control the process of heating and maintain the surface temperature of samples near the respective set points, indicating that these control schemes could be used to control microwave pasteurization of beef frankfurters.

Temperatures Measured by Fiber-optic Probe

Figure 6 shows the internal and surface temperatures measured by the fiber-optic probes as well as the corresponding surface temperature measured by the infrared sensor in each experiment. Because it took 10–15 min to attach a fiber-optic probe, the preparation procedure inevitably led to an increase in the sample temperatures. As a result, the initial temperature of the samples shown in Fig. 6 was higher than those shown in Fig. 5. The increase in the initial temperature of the samples also led to a decrease in the come-up time.

It shows in Fig. 6A that the surface temperatures measured by the infrared sensor and the fiber-optic probe agreed very closely. However, the temperature curves measured by the fiber-optic probe are smoother than those measured by the infrared sensor. Because the temperature set point was 75C, the measurement by both the infrared sensor and the fiber-optic probe indicates the surface temperature indeed stabilized around 75C in these experiments.

Although microwave heating is considered volumetric and is capable to heat from within, the heating of frankfurters is basically a net result of the penetration and absorption of microwave energy and the conduction of thermal energy. First the microwave energy penetrates through the surface into the interior of the frankfurters. The absorption of the microwave energy by frankfurters causes internal or volumetric heating. However, the intensity of the microwave power penetrating into frankfurters degrades exponentially as it travels from the surface into the interior (Padua 1993). In the meantime, conduction would occur to transfer heat from locations at a higher temperature to locations at a lower temperature. Depending on the thickness of the sample, either direct microwave heating or conduction may be a dominant mode of heat transfer. Therefore, the internal temperature of the frankfurters could not respond to the microwave power as fast as the surface temperature. Figure 6B shows the temperature histories measured at the geometric center of the five tightly packed frankfurters in the plastic packages. The temperature history curves at the geometric center show that heating of the interior could take a longer time even though the surface temperature can be quickly raised to a point lethal to *L. monocytogenes* and the heating by microwave is volumetric.

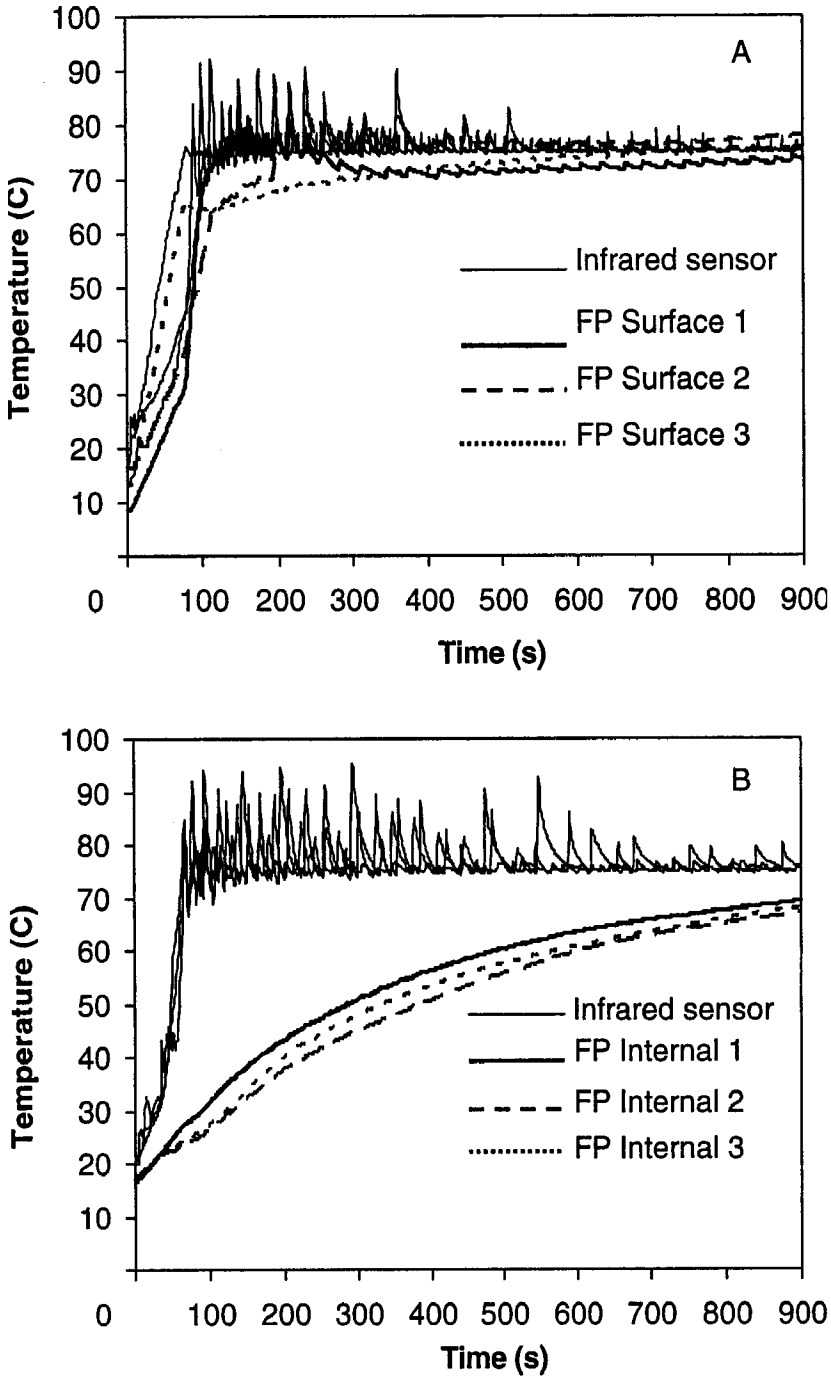

FIG. 6. THE SURFACE (A) AND INTERNAL (B) TEMPERATURES MEASURED BY FIBER-OPTIC PROBES DURING MICROWAVE HEATING
FP, fiber-optic probe.

Effectiveness of Microwave Pasteurization

Figure 7 illustrates the destruction of *L. monocytogenes* inoculated onto beef frankfurters during computer-controlled microwave pasteurization. The bacterial counts of control samples were plotted at time 0 when no microwaves were applied. For all the inoculated samples, the initial inoculum was between 6 and 8 log (cfu/g). After the surface temperatures of the samples were initially raised to set points, only 1–2 logs of bacteria were killed. Apparently, surface pasteurization was not sufficient to eliminate *L. monocytogenes* in beef frankfurters packaged in plastic bags because the internal temperatures at locations where frankfurters were tightly packed against each other were still low. The amount of microwave energies absorbed by the interior was not sufficient to kill *L. monocytogenes* at internal locations during the early stage of heating. Therefore, only a small portion of bacteria on the surface of samples might be killed at the early stage of microwave heating. In order to kill more bacteria, the surface temperature must be maintained to allow more microwave energy and thermal heat to travel into the interior of frankfurters.

After the surface temperature reached the set points, the bacterial counts of the beef frankfurters began to decrease with the holding time in approximately a linear manner. In fact, over the entire course of pasteurization by microwave, the bacterial counts decreased almost linearly with the heating time even though the surface temperature did not increase to the set points at the early stage of the heating.

Increasing the surface temperature only led to slight improvement in the effectiveness of bacterial destruction, but the overall heating time was not significantly shortened. Several factors may have contributed to this observation. First it took a longer time to reach higher surface temperature set points. Also the development of the internal temperature was not responding as fast as the surface temperature. This observation probably may be attributable to the uneven distribution of the microwave energies in the oven. Although the turntable in the microwave oven was used to minimize the uneven distribution of microwaves, it was still possible that the distribution of the wave patterns were still not perfect, leaving some cold spots in the samples. The overshooting during on-off control of the microwave oven also may have contributed to this observation. Understanding the distribution of microwave patterns in the microwave oven, however, was beyond the scope and purpose of this research.

In all experiments of this study, *L. monocytogenes* in beef frankfurters was destroyed in a fairly predictive manner. A 5-log reduction can be achieved if the samples were heated long enough (>10 min) in the microwave oven, although more than 7 logs of bacterial destruction was observed in the experiments. The level of bacterial contamination in frankfurters may be far less than 5 logs. Therefore, this technology could help the industry meet the minimum

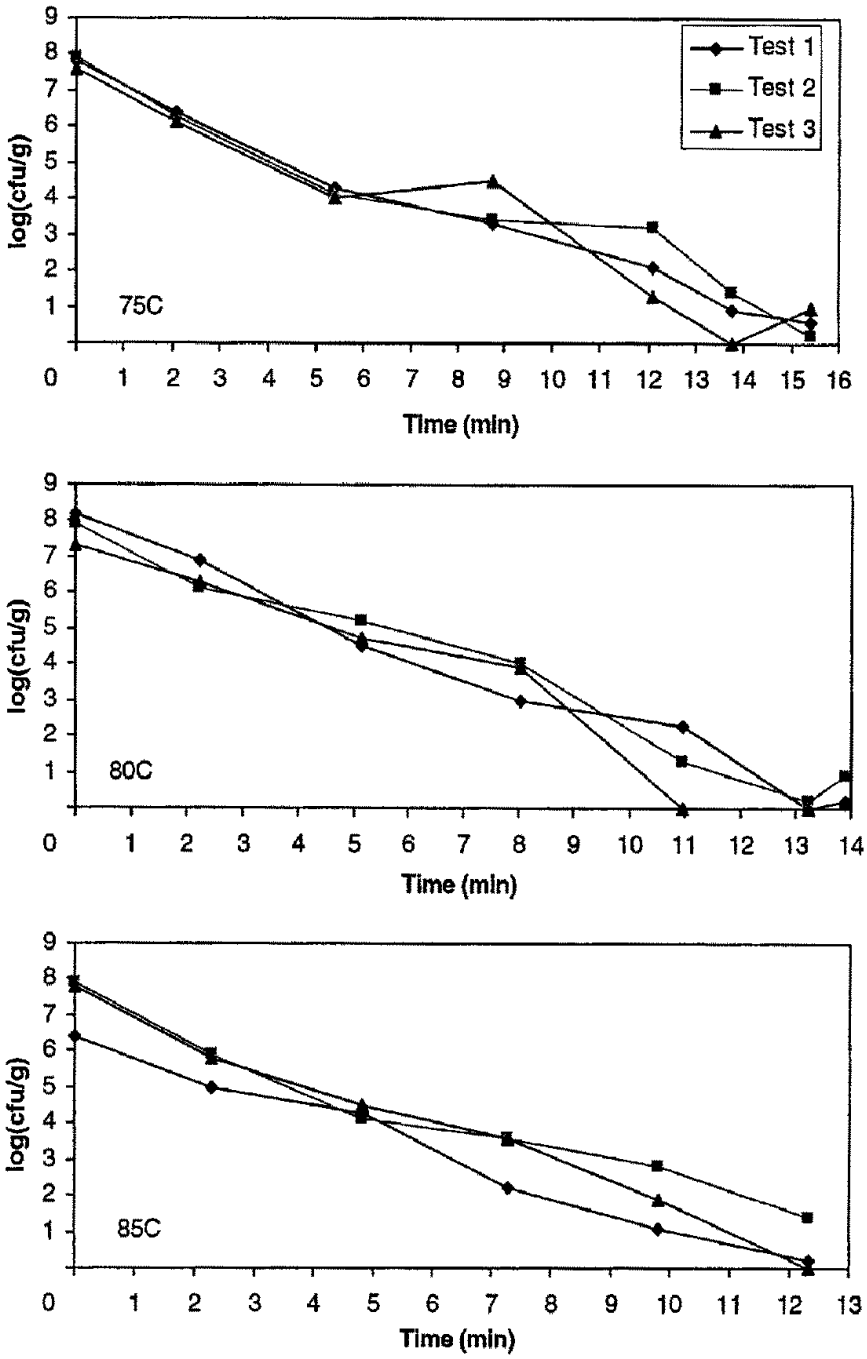

FIG. 7. EFFECTIVENESS OF COMPUTER-CONTROLLED MICROWAVE HEATING TO PASTEURIZE BEEF FRANKFURTERS AT 75, 80 AND 85C

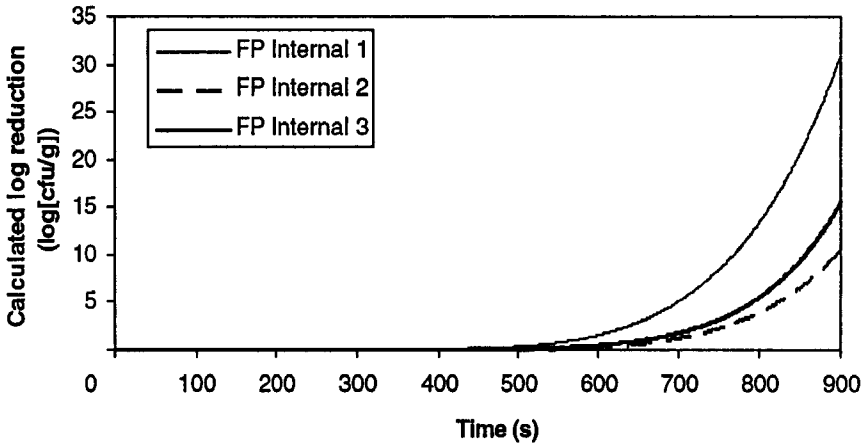

FIG. 8. TOTAL LOG-REDUCTION CALCULATED BASED ON THE TEMPERATURE MEASURED AT THE GEOMETRIC CENTER OF THE FRANKFURTER PACKAGES
The surface temperature set point was held at 75C.
Total heating time = 900 s (including come-up time).

requirements of 5-log reductions for thermal pasteurization of most perishable foods, including RTE meats. If this technology is used to treat RTE foods, they can be rendered free of *L. monocytogenes*.

Analysis of Thermal Processes

The temperature histories measured at the geometric center can be used to analyze the thermal processes using microwave heating, based on the thermal inactivation kinetics (Eq. 1). Figure 8 shows the total log-reduction calculated from the internal temperature curves shown in Fig. 6B with the surface temperature maintained at 75C. The temperature probe labeled as FP Internal 1 was probably not perfectly aligned at the geometric center of the package, and therefore the calculated lethality was a lot higher than those calculated according to the temperature histories measured by FP Internal 2 and 3. According to this figure, a 10- to 15-log reduction (based on FP Internal 2 and 3) can be achieved after 15 min of heating in the microwave oven with a surface temperature set point of 75C.

Because the surface temperature of frankfurters can be increased a lot quicker than the internal temperature at the center of the package, the microwave heating was effective in killing this organism on the surface of the package as soon as the surface temperature reached the lethal points. At the center of the package, however, the temperature of beef frankfurters did not respond to the microwave power as fast as the surface temperature, and

therefore it took a longer time for the heating to be effective in killing *L monocytogenes* at the geometric center of the frankfurters inside the package Overall, a 7-log reduction can be achieved in about 13–14 min of heating (based on the results of FP Internal 2 and 3). The calculated total lethality based on the center temperature histories measured by these two probes agreed fairly closely with the experimental observation (Fig. 7, 75C) where more than a 7-log reduction was achieved after 14–15 min of heating inside the microwave oven.

Effect of Microwave Heating on Products

After beef frankfurters were exposed to microwave heating, they experienced different changes in the physical attributes. The color was slightly darker than the control right after cooking, but it returned to normal after refrigerated storage. The length of beef frankfurters was reduced by approximately 10%, and the diameter was expanded by 10%. The change in the physical dimension of beef frankfurters may be attributed to the relatively long heating time experienced in this study. Because the power of the microwave oven used in the experiments was rated at only 550 W, which was one of the smallest microwave ovens available in the market, the amount of energy released from the magnetron was small. The long heating time can be substantially shortened if a more powerful oven is used because with a more powerful microwave oven, more microwave energy can be deposited on the product surface during each duty-cycle, and therefore more thermal energy can be absorbed by the products. The only reason for using such a small power microwave oven in this study was that it had simple mechanical controls and was very easy to modify. Almost all other modern models of household microwave ovens were equipped with sophisticated digital circuits or even self-protection mechanisms to prevent any modifications.

Although the total heating time for the in-package microwave pasteurization was relatively long (about 10 min), it was still substantially shorter than the processes using hot-water immersion or low temperature pasteurization (Murphy *et al.* 2001, 2003a,b; Muriana *et al.* 2002; Murphy and Berrang 2002a,b). This is probably because of the penetration of microwave energy into beef franks, causing them to heat from within.

Another approach to reduce the physical changes during in-package pasteurization of RTE meats is to use ovens with lower frequencies. The industrial microwave ovens usually operate at 915 MHz. Electromagnetic waves with 915 MHz can penetrate deeper into solid foods (Decareau and Peterson 1986). The power penetration depth also can be improved if even lower-frequency radio-frequency (RF) ovens are used. According to Wang *et al.* (2003), the penetrating power of RF waves (27 and 40 MHz) is almost 10

times longer than 2450-MHz microwave waves. In addition to the deeper penetration capability, the temperature distribution within solid foods is generally more uniform with RF heating. The more uniform temperature profile within solid foods could reduce or even eliminate cold spots during RF heating.

The efficiency of microwave/RF heating also can be improved with a better control mechanism, such a proportional-integral-derivative (PID) control mechanism. A PID mechanism can prevent the overshoot of the control temperatures on the surface of foods being treated in a microwave/RF oven, which in turn helps minimize the overheating caused by on-off control.

CONCLUSION

This study successfully tested the concept of in-package pasteurization of RTE meats (beef frankfurters) using computer-controlled microwave heating. This technology achieved more than 7 logs in the reduction of *L. monocytogenes* by raising the surface temperatures inside the plastic bags to and maintaining them at 75, 80 or 85C. The on-off control mechanism, although not perfect, was able to maintain the surface temperature of frankfurters around the set points. In all, this technology demonstrated its capability to inactivate *L. monocytogenes* in beef frankfurters within their final packages. If this technology is optimized, it can be used in the food industry to eliminate the risk of listeriosis caused by RTE meats.

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